Attorney Docket: 3002360-7040732001
(formerly 33154-176173)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	**Yang-Chang WU**		
Serial No.	**10/005,324**	Group Art Unit: **1625**	
Filing Date:	**December 7, 2001**	Examiner: **COVINGTON, Raymond K**	
For:	**CYTOTOXIC ANNONACEOUS ACETOGENINS FROM *ANNONA MURICATA***	Customer No. *23639* 23639 PATENT TRADEMARK OFFICE	
Atty. Docket No.	**AD7040852001 (formerly 33144-177127)**		

DECLARATION UNDER 37 CFR 1.132

I, Yi-Jen Lee, declare as follows:

1. I am an investigator at AdvPharma, Inc., located at No. 207, Hsia-Liao, Fu-An Village, Hou-Bi Tainan Taiwan (731) , Republic of China.

2. I have studied the cytotoxic effects of muricins A, E, and G in various human tumor cell lines. The muricins A, E, and G are extracted from *Annona muricata* and purified by the method described in the above-captioned patent application.

3. I used an MTT (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) metabolic assay method to test the muricins A, E, and G in five human tumor cell lines, including human colon adenocarcinoma (COLO205), human lung adenocarcinoma (H23), human acute lymphoblastic leukemia (MOLT-4), human prostate carcinoma (LNCaP), and an adriamycin-resistant human lung adenocarcinoma cell line (H23/0.3, overexpress p-glycoprotein).

4. The MTT method is fully disclosed by Moonks et al., J. Natl. Cancer Inst. (1991) 83(11),

1289-1290. In brief, the cells were seeded at a density of 6,000 ~ 10,000 cells into microtiter plates and incubated in 180μl culture medium overnight. Then, the cells were treated with muricins A, E, and G, respectively, at various concentrations for 72 hr. After treatment, viable cells were reacted with 20 μl MTT solution (5mg/ml) at 37°C for 4h. Then, 170 μl of the remainder, which contained the MTT-formazan crystals, was dissolved in 200 μl DMSO. Finally, the absorbance was measured at 545nm and reference at 690nm (Emax, Molecular Device Inc.). The dose-effect relationship of Muricins A, E, and G for antitumor activity was calculated by the formula: (Treatment OD_{545nm}-OD_{690nm} /Control OD_{545nm}-OD_{690nm}) x 100%. The IC_{50} (inhibition concentration) was determined between >50% and <50% inhibition by EXCEL software(Office 2000).

5. The results of my studies showed that muricins A, E, and G demonstrated tumor inhibition activities. Among them, Muricin A was potentially effective against LNCaP cells, the IC_{50} was 0.69 μM. Muricins G was potentially effective against COLO205 cells and an adriamycin-resistant lung tumor cells H23/0.3, the IC_{50} was 0.71μM and 1.55μM, respectively.

I hereby declare that all statements made herein true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Yi-Jen Lee

Yi-Jen Lee

May 6th, 2005

Date